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NONSPECIFIC STABILITY OF CELLS TO BACTERIAL TOXINS

The Concept of Cell Stress

[Following is the translation of an article by
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In 1957 Isaacs and Lindenmann established that in cells, under the influence of viruses which have entered them, a particular substance is formed which possesses an antiviral activity. The authors named this substance interferon, since it was primarily by its formation that it was possible to explain the long known phenomenon of interference, when a virus which has penetrated into a cell prevents the development of other viruses in it. The discovery of interferon served as the beginning for numerous investigations. All the investigated cultures of vertebrate cells turned out to be capable of developing interferon (Isaacs, 1961, 1963b). Its formation was also proven in the organism of animals and man (Baron and Bucier, 1963). The capacity to stimulate the development of interferon was established for any class of viruses. It was further revealed that it was inherent to many other agents - nucleic acids of various origin (Rotem et al., 1963), bacteria (Younger and Stinebring, 1964), bacterial endotoxins (Stinebring and Younger, 1964), and filtrates of certain molds (Kleinschmidt et al., 1964). By the present time this list in all probability is far from exhausted.

During the study of the physico-chemical properties of interferon its protein nature has been established. Its molecular weight fluctuates from 8,000 to 80,000. It has been noted that interferon-like substances, induced under the influence of non-viral agents, possess a greater molecular weight (50,000 - 80,000), while interferon obtained under the influence of viruses has the corresponding values of molecular weight of 10,000 - 30,000. Interferon is characterized by stability to low pH, relative thermostability, sensitivity to trypsin, and resistance to nucleases (Wagner, 1965).

The action of interferon is manifested both in respect to DNA-containing and RNA-containing viruses. It does not interact with the virus directly or with viral nucleic acid and does not influence their infectivity in vitro. The action of interferon is expressed indirectly through the cell. A characteristic feature of it is species specificity. The protective influence of

interferon is manifested on animals or in tissue cultures of the same species (Burke and Low, 1965).

At the present time the influence of interferon is viewed as a powerful factor of nonspecific antiviral immunity, promoting the recovery of an organism from the initial stages of development of viral infection. The majority of authors attribute to it a leading role in recovery during initial encounter with a virus (Baron, 1963; Isaacs, 1963a; Wagner, 1963, 1965).

If it is considered that interferon is developed by cells not only under the influence of viruses, but also of many other agents, bacteria and endotoxins for example (Stinebring and Youngner, 1964), then the question naturally arises whether or not the reaction of a cell to all these factors is limited by an increase of stability only to viruses. Is not the stated increase of stability a manifestation of a wider change in sensitivity of the cell developing under the influence of interferon. For checking this assumption in the present work, along with the challenge virus in the testing of stability of the cells we used diphtheria and staphylococcus toxins and also the endotoxin of *B. typhi*. Data on the increase of stability of cells to the diphtheria toxin under the influence of latent virus infection are presented in part in another work (Yabrov, 1966).

Materials and Method

Tissue culture. We used a 48-hour monolayer culture from tissues of 10--11-day old chick embryos. Since the inoculating dose comprised 5×10^5 cells in 1 ml, the cellular layer had already succeeded in forming by this time. As is known, in a culture of benign cells expressed growth of their numbers ceases as a result of contact inhibition after the formation of the dense monolayer. In this manner it was possible to additionally standardize the conditions of the experiment.

Toxins. We used untreated diphtheria toxin. Toxicity for guinea pigs comprised 700 Dlm in 1 ml. Endotoxin of *B. typhi* was obtained by the method of Raistrick and Topley (1934). The investigated preparation was very toxic for mice ($1 \text{ Dlm} < 0.125 \text{ mg}$). Both of these preparations were prepared by O. V. Savitskaya and O. M. Bodazhkovaya (LNIIVS). Staphylococcus toxin, the titer of which based on alpha-hemolysin comprised 1:512, was prepared by S. A. Anatoliy (Department of Microbiology, IEM). For the infection of cells we used a gradually increasing dilution of toxin. In order to be able to more accurately establish the dose of toxin corresponding to its titer, i.e., the ultimate dilution still causing the given degree of affection, the dilutions were conducted with a very small coefficient 0.1--0.2. Usually 3 test tubes were infected with each dilution. All told in each experiment we tested from 2- to 35 successive dilutions of toxin. The effect caused by

each of them was considered daily. Thus there was the simultaneous possibility to compare the cytopathogenic effect (CPE) on cells by doses of toxin which differed minimally from each other. For simplification in the tables are given only the titers of toxins, i.e., ultimate dilutions still producing the given degree of affection.

Results were considered based on the data of microscopic investigation of cultures which were infected by toxins. Microscopy was performed daily for 7--10 days. The CPE of toxin was designated depending on the degree of its expressiveness. Designations are presented in Table 1.

Viruses. For the production of interferon we used the louping ill virus in a quantity of 10^5 -- 10^6 tissue infectious doses. As the challenge we used the virus of vesicular stomatitis.

Interferon. As interferon we used the cultural fluid obtained after the 48-hour incubation of an infected culture of cells of a chick embryo. The control was the cultural fluid from a non-infected culture. The virus was inactivated by 60-minute heating at a temperature of 60°C . Antivirus activity of individual batches of interferon was expressed in titers of 1:32--1:128. For obtaining mouse interferon we used a primary monolayer culture from the tissues of mice embryos. The virus was neutralized with the help of homologous antiserum.

Results

The first tests were set up to establish whether or not the presence of interferon influences the sensitivity of cells to the cytopathogenic action of bacterial toxins. For this in some of the test tubes with the 48-hour monolayer culture from cells of chick embryo the growth medium was replaced with the supporting medium 199 with interferon ("test"). In the other test tubes with a culture of the same seeding control fluid was introduced in the appropriate dilution. The control fluid was obtained from non-infected cells of chick embryo ("control"). After incubation of these test tubes for a night at 37° the toxin was added to them and again they were placed in an incubator.

Table 1 presents the results of a study of the cytopathogenic effect of diphtheria toxin on cells of a monolayer culture of tissues of chick embryo in the presence of interferon. The results given are from individual tests on the 2nd or 3rd day after the infection. Distinct changes were observed on the 2nd day after introduction of the toxin. Absolute values of titers obtained in the different tests differed considerably among each other. However, a common feature for all the tests is the fact that the titers of toxin, corresponding to a specific degree of affection in the culture, in the presence of interferon were invariably lower in comparison with the same indices in the control. In certain tests this divergence is expressed especially sharply in relation to doses of toxin which cause maximum affections of the cellular layer.

Table 1

Cytopathogenic effect of diphtheria toxin on cells of a tissue culture of chick embryo in the presence of interferon

1		2		3		4		5		6		7		8		9	
Cytotoxicity		Interferon		Toxin		Control		Toxin		Control		Toxin		Control		Toxin	
++		1000		2000		5500		10000		10000		2500		1000		2000	
++		3000		4000		6000		12000		12000		4000		3000		4000	
+		5000		5500		7000		13000		10000		7000		4000		5000	

Notes: Conditional designations for the degree of affection of cells by toxin:
 + - granularly of protoplasm, rounded cells present, completeness of cellular layer not disrupted; ++ - along with changes in the cells there are disruptions of the completeness of the cellular layer; +++ - disconnected changed cells, layer is absent;
 — - changes in the stated degree of expressiveness absent. Figures in the table designate the reverse value of the titer of toxin, i.e., its maximum dilution, capable of causing the corresponding affection of cells.

Key: (a) Degree of affection of cells; (b) Cytopathogenic effect of toxin in the presence of interferon and in the corresponding control in various tests; (c) test; (d) control.

Thus, in tests 1, 4, 7, and 8 in the presence of interferon in order to cause a reaction which was designated +++ it was necessary to use doses of toxin exceeding by 4-5 times the amounts which were sufficient for this in the control (200 and 1000, 1500 and 5500, 700 and 2500, 1000 and 4000 in the test and in the control correspondingly). In other tests this divergence was approximately the same for each of the threshold doses. Thus in tests 2, 3, and 9 for all three doses, corresponding to a specific degree of CPE, the divergence in the test and in the control is 2--3-fold, or, as in tests 5 and 6, 1.3--1.5-fold.

Since the effect of toxin on the cells did not cease even after the appearance of the first affections, in the subsequent days the development of a cytopathogenic effect was observed. It was manifested in an increase of titers of toxin which corresponded to the specific degree of affection. During observation of such infected cultures for 7-10 days it was possible to note that this increase reaches a specific limit. Thus it was possible to expose an ultimate threshold dose of toxin which was capable of causing the corresponding affections in a culture during maximum exposure. The results of such a prolonged observation of the cells in an example of one of the tests (test 3) are presented in Table 2.

Table 2

Dynamics of cytopathogenic changes caused by the action of diphtheria toxin on cells of a tissue culture of chick embryo in the presence of interferon

a Сте- пень пора- жения клеток	b Время после заражения (сутки)											
	2		3		4		5		6		9	
	c опыт	d конт- роль	c опыт	d конт- роль	c опыт	d конт- роль	c опыт	d конт- роль	c опыт	d конт- роль	c опыт	d конт- роль
+++	600	1000	1000	2000	1500	3000	1500	5500	1500	5500	4000	8000
++	800	2000	1000	3000	2000	5500	5000	6000	5000	8000	3500	—
+	1000	3000	2000	5500	4000	—	—	6500	—	—	—	—

Note: Conditional designations are the same as in Table 1.

Key: (a) Degree of affection of cells; (b) Time after infection (days); (c) test; (d) control.

It can be seen that the condition of increased resistance of culture cells to diphtheria toxin in the presence of interferon in comparison with the control is preserved throughout the entire period of observation. It is expressed first of all in the retained differences in the titers of toxin which cause the corresponding cytopathogenic effect in the control and in the test. Dilutions of

toxin which cause, for example, an expressed affection of cells corresponding to ++, in the presence of interferon remain constantly lower (1:800, 1:1000, 1:1500, 1:5000, 1:5000, and 1:5500) in comparison with the control (correspondingly 1:2000, 1:3000, 1:5500, 1:6000, 1:9000, and 1:9000). The increased stability of cells in the test testifies to a later, in comparison with the control, manifestation of symptoms of affection with the same dose of toxin. Thus, in a dilution of 1:4000 the toxin caused a minimal (+) affection of cells in the control on the 2nd day after its introduction. In the presence of interferon the initial changes, corresponding to the same dose of toxin, were observed only on the fourth day. The high degree of protection of cells in the presence of interferon is testified by the expressed difference between the test and the control in titers characterizing the threshold of sensitivity which is established during the prolonged action of the toxin. If in the control the extreme dilution of toxin capable of causing the affection of culture cells on the 9th day after infection comprised 1:9000, then in the test it was equal to 1:5500.

Table 3

Cytopathogenic effect of staphylococcus toxin on cells of tissue culture of chick embryo in the presence of interferon

Сте- пень по- врежде- ния клеток	Цитопатогенное действие токсина в присутствии интерферона и в соответствующем контроле в разных опытах															
	10		11		12		13		14		15		16			
	опыт	контр- роль	опыт	контр- роль	опыт	контр- роль	опыт	контр- роль	опыт	контр- роль	опыт	контр- роль	опыт	контр- роль	опыт	контр- роль
+++	4.0	4.5	3.5	3.7	4.0	4.7	3.0	3.7	3.2	6.5	4.3	5.6	3.8	4.1		
++	7.0	12.0	4.7	6.6	5.5	7.3	12.0	18.0	12.0	19.0	5.2	7.3	4.7	6.0		
+	12.0	24.0	5.7	8.2	6.9	8.2	16.0	24.0	16.0	24.0	6.0	9.0	6.9	8.7		

Note: Conditional designations are the same as in Table 1.

Key: (a) Degree of affection of cells; (b) Cytopathogenic effect of toxin in the presence of interferon and in the corresponding control in various tests; (c) test; (d) control.

Table 3 presents the results of analogous tests conducted with the use of staphylococcus toxin. This toxin exerted a cytopathogenic effect on cells only in small dilutions. The titers of it, corresponding to maximum (+++) and minimum (+) changes in the cells, usually did not exceed indices from 1:6.5 to 1:32. In order to be able to observe the minimum differences in absolute values in the test and in the control we used small intervals in the dilution of toxin. From 1:2 to 1:10 the differences between successive dilutions of toxin used for infection did not exceed 0.2--0.4.

Subsequent dilutions usually differed by a unit. The results were considered on the following day after infection.

It is apparent that in the presence of interferon the cells turned out to be more resistant to the cytopathogenic effect of staphylococcus toxin in comparison with the control cells. In each of the tests the toxin caused the corresponding harm in these cells, only being taken in lesser dilutions. The differences in titers were not as significant as in the case with diphtheria toxin. However, they constantly had one and the same nature: in all the tests the dilutions of toxin which caused damage to cells in the presence of interferon were lower than the corresponding dilutions in the control. Stemming from the criterion of signs it is easy to determine that the probability that these differences are not accidental exceeds 0.99.

During the dynamic study of the cytopathogenic effect of staphylococcus toxin it was possible to note a specific peculiarity. The CPE was distinctly expressed in the morning of the day following infection, i.e., in 20-24 hours. During the course of the day the changes in the cells increased, and by evening it was possible to note a further distinct shift in titers. However, on the 2nd day after introduction of the toxin the increase in the cytopathogenic effect ceased. On the contrary, repair was observed. The clearest reverse changes were expressed in test tubes where the minimum damaging doses of toxin were introduced, and in which damages were less expressed. It is necessary to note that cultural fluid, taken from a test tube where upon microscopic examination complete restoration was noted, exerted a toxic effect when introduced into a fresh culture. Thus the disappearance of initial damages cannot be explained by the inactivation of the toxin which was introduced.

Table 4 presents the results of observations in the dynamics of one of the tests (test 11). There is an apparent increase of cytopathogenic effect during the first 24 hours after infection. At the end of the first day the corresponding damages in the cells are caused by lesser doses of toxin. Here the difference in the sensitivity of the cells which was noted earlier is preserved. Cultures which contained interferon turned out to be less sensitive in comparison with the control. On the 2nd day both in the test and in the control a distinct weakening of cytopathogenic effect is noted. In cultures where a night prior to this changes were determined by indices of +++ and ++, the same doses of toxin corresponded to ++ and +. In test tubes where changes were minimal (+) from the very beginning by the 3rd day they had quite disappeared. Since the effect of the toxin did not cease, then on the 4th day the signs of damage were manifested again in all the test tubes. It is apparent that the indices of cytopathogenic activity of toxin approximate the initial ones observed in the first 24 hours. And, finally, on the 5th day there is a sharp increase of damaging effect, testifying to a secondary (Aleksandrov, 1965) significant disruption

of compensator mechanisms in the cells. Again it is necessary to note that during the prolonged effect of staphylococcus toxin in the presence of interferon the cultures preserved a higher stability to toxin in comparison with the control.

Table 4

Dynamics of cytopathogenic changes caused by the action of staphylo-toxin on cells from a tissue culture of chick embryo in the presence of interferon

a Степень пораже- ния кле- том	b Время после заражения (сутки)											
	1 (утро) c		1 (вечер) d		2		3		4		7	
	e	конт- роль	e	конт- роль	e	конт- роль	e	конт- роль	e	конт- роль	e	конт- роль
	опыт	опыт	опыт	опыт	опыт	опыт	опыт	опыт	опыт	опыт	опыт	опыт
+++	3.5	3.7	4.0	4.7	3.5	3.7	3.5	4.2	4.0	5.4	7.2	9.4
++	4.7	6.6	5.5	7.3	4.0	5.4	4.5	5.4	4.7	6.9	8.7	>11.0
+	5.7	8.2	6.9	8.3	5.3	6.6	—	—	6.9	9.0	>11.0	—

Note: Conditional designations are the same as in Table 1.

Key: (a) Degree of damage to cells; (b) Time after infection (days); (c) morning; (d) evening; (e) test; (f) control.

Table 5

Cytopathogenic effect of endotoxin of B. typhi on cells of chick embryo tissue in the presence of interferon

a Сте- пень пора- жения	b Цитопатогенное действие эндотоксина в присутствии интерферона и в соответствующем контроле в разных опытах							
	17		18		19		20	
	c	конт- роль	c	конт- роль	c	конт- роль	c	конт- роль
	опыт	опыт	опыт	опыт	опыт	опыт	опыт	опыт
+++	2.0	4.0	2.0	8.0	<4.0	4.0	3.0	5.0
++	3.0	16.0	8.0	24.0	4.0	14.0	7.0	14.0
+	4.0	24.0	12.0	32.0	7.0	22.0	11.0	24.0

Note: Conditional designations are the same as in Table 1.

Key: (a) Degree of damage; (b) Cytopathogenic effect of endotoxin in the presence of interferon and in the corresponding control in various tests; (c) test; (d) control.

Under analogous conditions tests were made of the influence of the presence of interferon in a culture on the cytopathogenic effect of B. typhi. As can be seen from the data in Table 5, in this case

also the protective effect of interferon was manifested quite clearly. Dilutions of toxin, causing the corresponding changes of cells in the test, were 2-6 times lower in comparison with the control. Thus the CPE, determined as ++, in the test and in the control corresponded to titers of 3 and 16, 8 and 24, 4 and 14, and 7 and 14 (tests 17-20).

Thus the data presented testify that in the presence of interferon the cells of a monolayer culture of chick embryo turned out to be more resistant to the cytopathogenic action of diphtheria and staphylococcus toxins, and even to the endotoxin of *B. typhi*.

In studying the mechanism of antiviral activity of interferon, Isaacs and Lindenmann (1957) established that interferon acts not on the virus, but on the cell, making it resistant to the virus. Preliminary exposition of viruses with interferon does not lower their infectious activity. If interferon is removed after a day, or even several hours, after its administration into a culture, the cells preserve a resistance to virus for several days.

In order to clear up whether or not interferon exerts a direct inhibiting effect on toxin the following tests were set up. To 0.9 ml of undiluted interferon, the titers of which comprised 1:64 and 1:128, 0.1 ml of diphtheria toxin was added (I). In the control the toxin was introduced into medium 199 (II). After a 24-hour exposure at 35° the stated mixtures, and also the initial toxin which had been stored at 4° (III), in dilutions from 1:1000 to 1:10,000 were introduced into a culture. Considering that the titer of interferon used in the test did not exceed 1:128, its protective action on cells with such a test set-up could not be manifested. Thus the weakening of toxin in test I, if it took place, could be explained by the direct inhibiting action of interferon. However, the indices of activity of toxin in tests I and II turned out to be practically identical (Table 6). The certain weakening of activity of toxin in tests I and II in comparison with initial (III) apparently is explained by the influence of the temperature of 35° (tests 21 and 22).

Tests were also set up to establish whether or not the state of increased resistance of cells to the cytopathogenic action of bacterial toxins is preserved even after removal of interferon. In contrast to the previous tests the interferon was removed on the following day after its introduction. The cells were washed with 2 ml of Hanks solution, 1 ml of medium 199 was added, and then they were infected with toxin.

The results of these tests are presented in Table 7. It can be seen that cells which were subjected to the preliminary treatment with interferon were more resistant to the effect of bacterial toxins in comparison with the control. For each of the toxins the doses, causing a specific degree of damage in the culture, were constantly higher in the test (tests 23-33). During dynamic observations it was easy to establish that other features of increased

stability, revealed in tests 1--20, were also preserved (later manifestation of damage with the same dose of toxin, difference in the levels of threshold sensitivity).

Table 6

Study of the direct influence of interferon on the cytopathogenic activity of diphtheria toxin under conditions of daily contact at a temperature of 35°

Степень поре- жения клеток	Опыт 21			Опыт 22		
	I	II	III	I	II	III
+++	2500	2500	3500	1500	2000	2500
++	3500	3000	5500	2500	2500	5000
+	4500	4500	6500	4000	4500	6000

Note. I--III - test variants: I - mixture of toxin with interferon, II - mixture of toxin with medium 199, III - initial toxin, stored at a temperature of 4°. Remaining designations are the same as in Table 1.

Key: (a) Degree of damage to cells; (b) Test 21; (c) Test 22.

As is known cells which are infected with a latent virus, which during its multiplication does not exert a cytopathogenic effect on them, display a stability to superinfection due to the constant development of interferon by them. The phenomenon of interference is used for the exposure of a latent virus infection in a culture. Introduction into such a culture of another - challenge - virus, which during multiplication displays a distinct cytopathogenic effect, may, based on the suppression of this effect, establish the presence of a latent infection.

It was also interesting to study if there was an increase in the stability of cells in a culture, infected with latent virus, to the action of bacterial toxins. The tests were set up in the following manner. 2-3 day cultures were infected with the louping ill virus with a calculation of 10^4 tissue infecting doses in 1 ml of medium 199. The control test tubes received 1 ml each of medium 199 without the virus. After a 2-day incubation at a temperature of 37° the titer of interferon in the liquid phase of infected cultures in the various tests reached indices from 1:16 to 1:64. These cultures were resistant to the action of a challenge virus of vesicular stomatitis, introduced in a quantity of 10^5 - 10^6 tissue cytopathogenic doses. Parallel with resistance to virus a test was made of the resistance of these cultures to toxins. For this, 2 days after infection with louping ill virus successive dilutions of toxin were introduced

into them. At the same time control, non-infected cultures were inoculated with toxin.

The results of these tests are presented in Table 8. In tests 34-38, where diphtheria toxin was tested, cells preliminarily infected with louping ill virus turned out to be more resistant. It is apparent that in all cases the doses of toxin, causing a specific degree of damage in infected cultures, were constantly higher in comparison with the control.

The same result was also obtained during the testing of endotoxins of *B. typhi* (tests 39-41). Just as in the case with diphtheria toxin, the endotoxin exerted a cytopathogenic effect on cells which were preliminarily infected with virus, but taken in lesser dilutions than in the control. However, it is necessary to note that cultures infected with louping ill virus turned out to be less protected in respect to the cytopathogenic effect of toxin than non-infected cultures, into which the interferon was introduced from without. It was especially noticeable during prolonged observation of the action of toxin on infected cells. Though differences were preserved here in the threshold sensitivity in comparison with the control, they were expressed more weakly. This can be explained by the fact that the latent virus, though it does not exert a cytopathogenic effect on the cells, still causes sharp shifts in their metabolism, energetic in particular (Yabrov et al., 1964; Golubev, 1966). In the process of virus reproduction the membrane permeability of the cells is disrupted (Golubev et al., 1965). Apparently in spite of the development of interferon, changes caused by the presence of latent infection reflect unfavorably on the compensator capabilities of the cell by lowering its resistance, which in the examples presented above was manifested more distinctly during the prolonged influence of toxin on an infected culture.

As can be seen from Table 8 (tests 42-44), an especially sharp negative influence of current latent infection on the resistance of cells to the effect of toxin was manifested during the testing of staphylococcus toxin. Cells, preliminarily infected with the louping ill virus, turned out to be more sensitive to the cytopathogenic effect of staphylotoxin than control non-infected cells. Reverse changes, which in the previous tests were noted as characteristic for the cytopathogenic effect caused by staphylococcus toxin, in the tests of this series were observed only in control non-infected cultures.

One of the characteristic properties of interferon, noted by the majority of authors, is its species specificity. In order to clear up if species specificity, which is so characteristic for the antiviral protective effect of interferon, is also preserved in the case of bacterial toxins, a test was set up in which, along with chicken (I), interferon was used which was obtained on a primary

Table 7

Influence of preliminary interferon treatment of cells of a monolayer culture of chick embryos on their sensitivity to the cytopathogenic effect of bacterial toxins

Система маркиро- вания фла- гов	(a) Дифтерийный токсин												(b) Цитопатогенный токсин												(c) Эндоотоксин D. typhi											
	23			24			25			26			27			28			29			30			31			32			33					
	опыт	контр- роль	б	опыт	контр- роль	б	опыт	контр- роль	б	опыт	контр- роль	б	опыт	контр- роль	б	опыт	контр- роль	б	опыт	контр- роль	б	опыт	контр- роль	б	опыт	контр- роль	б	опыт	контр- роль	б						
+++	1500	3500	2000	5500	900	3500	5000	7500	4.0	4.2	<3.5	3.5	4.5	5.7	10	15	20	<20	20	40	100	15	50	80	20	40	100	15	50	80	<20	20	40	100		
++	3000	4500	5500	8000	3500	6500	8000	15000	7.0	10.0	4.0	6.2	5.7	7.7	15	30	40	20	20	40	100	15	50	80	20	40	100	15	50	80	20	40	100			
+	4000	6000	6500	10500	4500	11500	10000	27000	11.0	16.0	6.2	7.7	7.2	9.4	30	60	100	20	20	40	100	15	50	80	20	40	100	15	50	80	20	40	100			

Note: Conditional designations are the same as in Table 1. In test 33 a 24-hour culture was used which was more sensitive to the cytopathogenic effect of endotoxin. Key: (a) Degree of damage to cells; (b) Diphtheria toxin; (c) Staphylococcus toxin; (d) Endotoxin of B. typhi; (e) test; (f) control.

Table 8

Influence of latent infection, caused by the louping ill virus, on the sensitivity of cells of a culture of chick embryo to the cytopathogenic effect of bacterial toxins

Степень пораже- ния на- тои	Дифтерийный токсин										Эпидемический В. тифи										Стафилококковый токсин									
	34		35		36		37		38		39		40		41		42		43		44		45		46		47		48	
	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль	опыт	контр-роль
+++	1000	3000	3000	9000	1000	5000	<1000	1000	200	600	<4.0	4.0	2.0	5.0	<4.0	4.0	3.7	3.2	5.6	3.3	5.8	5.3	5.6	3.3	5.8	3.3	5.6	3.3	5.8	5.3
++	6500	8000	7000	12000	3500	7000	1000	3000	500	1500	<4.0	6.0	3.0	8.0	<4.0	8.0	5.6	3.7	7.3	7.3	9.8	7.0	7.3	9.8	7.3	7.3	9.8	7.0	7.3	7.0
+	—	9000	8500	16000	4000	10000	—	—	800	3500	4.0	9.0	4.0	10.0	<4.0	10.0	7.3	4.7	11.5	7.3	11.0	8.2	11.5	7.3	11.0	7.3	11.5	7.3	11.0	8.2

Note. Conditional designations are the same as in Table 1.
Key: (a) Degree of damage to cells; (b) Diphtheria toxin; (c) Endotoxin of B. typhi; (d) Staphylococcus toxin; (e) test; (f) control.

monolayer culture from tissues of mouse embryo (III). Just as in the previous tests, the protective effect of chick interferon is apparent (Table 9). In its presence the titers of diphtheria toxin, causing a specific degree of damage to cultures, was 2-4 times lower in comparison with the control. At the same time it was not possible to reveal an increase in the stability of cells from a monolayer tissue culture of chick embryo to the action of diphtheria toxin when interferon was introduced which was obtained on a culture from mouse tissue. In the presence of mouse interferon the CPE of diphtheria toxin on chick cells was manifested in the same doses as in the control. Thus the gradually increasing degree of affection in the control and in the test tubes with mouse interferon on the 2nd day after infection was matched by titers of toxin equal to 4 and 5; 3, 5, and 4; 3 and 2, 4. These relations were preserved for the entire period of observation.

Table 9

Cytopathogenic effect of diphtheria toxin on cells of a tissue culture of chick embryo in the presence of mouse interferon

a) Степень поражения клеток	b) Время после заражения (сутки)								
	2			3			6		
	I	II	III	I	II	III	I	II	III
+++	800	3000	2500	1000	4000	4000	2000	15000	10000
++	1500	3500	4000	2500	7000	6500	2500	—	14000
+	2000	4000	5000	3000	8000	8000	4500	—	—

Note: I--III - test variants. I - 24 hours prior to infection chick interferon was introduced into the test tubes, II - control to I, III - mouse interferon was introduced 24 hours prior to infection. The remaining designations are the same as in Table 1.
Key: (a) Degree of damage to cells; (b) Time after infection (days).

Discussion

The investigations of interferon were devoted to a study of it as a factor of non-specific antiviral immunity. In using substances of a various nature for its development, the authors determined the development of a change in the sensitivity of cells of a tissue culture or organism to the infectious activity of viruses. In the opinion of Wagner (1965) a unique method for the identification of interferon at the present time is its capacity to inhibit the multiplication of virus.

A principal difference in the results which were obtained in the present work was that they testify to a wider spectrum of resistance developing in the cells under the influence of interferon. It was shown that under conditions which lead to the development of virus interference the cells manifested an increased resistance not only to the virus, but also to the cytopathogenic effect of diphtheria and staphylococcus toxins and also to the endotoxin of *B. typhi*. A substance which protects the cell from the effect of toxins was developed under the influence of virus, its activity was preserved after heating for an hour at a temperature of 60°, and it was higher in tests which displayed a more expressed anti-virus effect. Just as in the case with challenge virus, it did not exert a direct inhibiting effect on the damaging agent, which was bacterial toxin. Available results testify in favor of the presence of species specificity of its action. All these findings make it possible to identify the substance, responsible for increasing the resistance of cells to the CPE of bacterial toxins, with the already known antiviral interferon.

Certainly one cannot exclude the possibility that under the influence of virus in the cell along with the substance which ensures its resistance to viruses a substance (or substances) is developed, or manifests its activity, which is capable of increasing resistance to other influences beside viral. However, this proposal does not change the essence of the fact that in the process of development of interferon under the influence of virus there is an increase of resistance of the cell not only to viruses, but also to the action of completely different agents, such as bacterial toxins. And further, the product formed here is able to impart the stated condition of non-specific resistance to other cells which had not been subjected to the action of the virus. Thus the problem amounts to whether or not the state of increased resistance to various agents under the influence of interferon is ensured as a result of the action of one substance - interferon itself, or is this substance complex and do its components ensure the resistance of cells to various influences. Special investigations are necessary for solving this problem. Until this we consider it feasible to use the term "interferon" for designating substances which ensure a state of non-specific resistance in cells which develops under conditions connected with the phenomenon of interference, regardless of under which influences on the cell this resistance is manifested.

As was already mentioned, the numerous findings which have accumulated by the present time testify that interferon is revealed in cells not only under the action of viruses, but also many other substances - heterologous nucleic acids and their derivatives, polysaccharides of bacteria and molds, etc. Their number is constantly

increasing. Thus, under the influence of certain stimuli a condition of increased resistance to the action of various agents emerges in the cell. These agents are, on the one hand, intracellular parasites - viruses, and on the other hand - bacterial toxins. In all probability this interference is not limited to the cited examples, and the phenomenon of interference of viruses is only a partial manifestation of it. This condition is the result of a complex and, to a certain degree, similar mechanism of reaction of protein transformations which are coded by nucleic acids (Heller, 1963; Taylor, 1964; Levine, 1964; Ho and Breinig, 1965).

Data have been obtained that interferon-like substances, forming during the action of non-virus agents on a cell, already exist in it, being found in a nonactive state under the influence of repressors, or in compounds which are denatured under the action of the stated stimuli (Wagner, 1965). Consequently, changes originating in the cell during the process of development of interferon, and further under its influence leading to the emergence of a condition of increased resistance, are found in conformity with the protein theory of stimulation, according to which the response reaction is determined primarily by the transformations of proteins which are taking place in it (Nasonov and Aleksandrov, 1948). The fact that they are regulated by nucleic acids, in our opinion, only specified a possible route for the stated transformations in connection with the expansion of our knowledge concerning the regulating role of nucleic acids in the dynamics of cell proteins.

It is necessary to note that a similar response to the action of various agents is characteristic for a living cell (Nasonov and Aleksandrov, 1940) and, in all probability "advantageous," since in particular it makes it possible to mobilize protective mechanisms even under the influence of unusual stimuli which have not been encountered earlier (Aleksandrov, 1948; Aleksandrov and Feldman, 1958). At the same time, based on presently available information, it can be assumed that the adaptation reaction examined by us has specific peculiarities: though it emerges under the action of various substances, a common property for them is the capacity to "turn on" the mechanism of interferon formation. It is primarily this mechanism, representing a specific sequence of protein transformations originating under the control of nucleic acids of the cell, which is the other characteristic feature of this reaction. In the course of this process the formation of interferon, a mediator which is able to impart a state of resistance to intact cells without the participation of the agent which caused its formation, also distinguishes the reaction being considered. In connection with the stated peculiarities and harmony of mechanisms lying at its base, it is expedient to discriminate these conditions under the general name of "cell stress." The interference of viruses is a partial manifestation of this condition.

The protein theory of stimulation provides for the possibilities of specific peculiarities in the reaction of a living cell to various stimuli, which may be connected with their elective action on cell structures which are different in functional designation and sensitivity (Nasonov and Aleksandrov, 1940, p 211). The condition of cell stress is, in our opinion, an example of such a reaction of a cell, the non-specificity of which is relative. At its basis lies a similarity in the site of application and, possibly, in the mechanism of action of various stimuli, the result of which is the specific nature of the reaction of the cell, common for the given effects and at the same time specific for them, which is inherent to it. The separation of similar conditions on the basis of their determining mechanisms and isolated study within the framework of the common adaptation concept (Aleksandrov, 1948, 1956, 1965), based on the protein theory of stimulation (Nasonov and Aleksandrov, 1940; Aleksandrov, 1948), should return a specific profit. It makes it possible to draw certain phenomena closer and to group the facts which have been accumulated, and to facilitate their understanding and open the possibility for a united methodical approach for their subsequent investigation, which in the final analysis brings closer and facilitates the practical use of the data obtained.

The name "cell stress" has the advantage that the term "stress" at present has become generally accepted for designating a complex monotypic non-specific adaptation reaction with a wide spectrum of resistance, developing in response to various stimuli. It is necessary to note that the mechanism of development of stress in an organism cannot be reduced to a reaction in cell tissues. There are data that the condition of stress (stress - Selye, 1950) of an organism in its narrow understanding as a reaction, connected with an increase of activity primarily of the hypophysis-adrenalin system, is characterized by the suppression of activity of the reticuloendothelial system, which is at present connected with the development of interferon in an organism (Ho, 1964). In working with mice which were in a state of stress, Chang and Rasmussen (1955) recently showed that a lowering of their capacity for the development of interferon took place under the influence of the virus of vesicular stomatitis.

At the present time interferon itself is viewed only as an intermediate product in the process of formation of non-susceptibility of cells to viruses (Taylor, 1964). The possibility is not excluded of the existence in nature or the artificial synthesis of substances which are able to stimulate the course of the end phase of this process, but passing by the formation of interferon. In the opinion of a number of investigators the resistance to viruses, developing in cells under the influence of nucleic acids of a non-virus origin, is not connected with the development of interferon. The increase of resistance is observed only in the presence of the stated substances and is lost after washing of the cells. Interferon is not

revealed in the pericellular fluid of such cultures. Based on our own observations under specific conditions, in particular with the help of certain low-molecular substances, it is easy to cause a significant increase of the resistance of cells of both primary and transplanted cultures to the cytopathogenic effect of viruses and bacterial toxins, apparently without the participation of interferon. Subsequently it may turn out useful to apply the term "cell stress" in a wider sense for defining a state of increased non-specific resistance of cells to various unfavorable influences, considering here that it may take place without the participation of interferon.

As is known, interferon is developed not only in tissue cultures but also in the organism (Wagner, 1965, and others). Keeping in mind the fact that practically all the results obtained up until the present time in the study of interferon in tissue cultures have been confirmed in tests on animals, it is necessary to check the possibility that in an organism the resistance of cells which emerges with its participation also has a wide spectrum, i.e., in an organism various stimuli can cause a condition of cell stress. With this it may be possible to obtain an acceptable explanation of certain facts which are not completely clear. Thus it has been known for a long time that in response to the administration of endotoxin into an organism a resistance emerges not only to that endotoxin but also to other endotoxins which do not have an antigenic similarity with it. On the other hand, it has been reliably proven at present that endotoxins, when introduced into an organism, are able to cause the development of interferon, the activity of which in these investigations was tested based on the inhibiting effect on viruses (Stinebring and Youngner, 1964). If it is possible to establish here that the developing condition of cell stress is characterized, in particular, by an increase of resistance of the cells to the action of endotoxins, then the above cited fact of the non-specific cross resistance of endotoxins may be explained by the protective influence of interferon. Based on our data, after the administration to mice of the virus of Newcastle disease or of homologous interferon, synthesized under the influence of the virus, a distinct increase is noted in the resistance of animals to the lethal effect of endotoxins of *B. typhi*. The antitoxic action of interferon in an organism is reported by Nemes and Helleman (1962). When mice were given endotoxin they were able to suppress not only the infectious activity, but also the neurotoxic effect of a concentrated preparation of influenza virus. The same explanation is also applicable for a share of the phenomena connected with the non-specific protective action on an organism by pyrogens, the majority of which are a component part of endotoxins (Tovarintskiy, 1947). The possibility of the emergence of the stated condition of non-specific resistance should also be considered during other infectious

processes, not connected with the participation of viruses. At the present time there are data concerning the development of interferon following the administration of bacteria and rickettsia to an organism (Youngner and Stinebring, 1964). Not excluded is the possibility of formation in an organism of products, stimulating the development of interferon following the aseptic inflammation or immunological reactions, as this takes place with the formation of endogenic pyrogens. In this connection attention is drawn to the specific similarity in the properties of leukocytic endogenic pyrogen and interferon (protein nature, sensitivity to proteolytic enzymes, relation to temperature, species specificity, etc. - Sorokin, 1965). Also not excluded is the possibility that the developing resistance of cells is not limited to the action of infectious agents, which viruses are, or of bacterial toxins. All these problems require solving.

Summary

At the present time the development of interferon is viewed only as a manifestation of non-specific antiviral immunity. At the same time numerous findings testify that interferon is developed following the action on cells of many other substances of a non-virus nature. This fact makes it possible to assume that antiviral activity of interferon is a manifestation of a wider change of resistance which takes place with its participation.

Results obtained testify in favor of this proposal. It has been established that in the presence of interferon there is an increase of stability of cells from a culture of chick embryo to the cytopathogenic effect of diphtheria and staphylococcus toxins, and also the endotoxin of *B. typhi*. This increase of stability was also preserved after removal of interferon. Interference of virus was also obtained with the cytopathogenic action of diphtheria toxin and the endotoxin of *B. typhi*. It has been established that interferon does not exert a direct inhibiting effect on toxin in vitro. Its activity, just as during the action of viruses, is expressed indirectly through the cell.

In the work the proposal is put forth that the development of interferon is the manifestation of the adaptation reaction of the cell, as a result of which an increase of stability to various stimuli emerges. The reaction of interference of viruses is only its partial manifestation. Processes, leading to the emergence of the stated condition, are characterized quite sufficiently from the position of the protein theory of stimulation, according to which the nature of the response reaction of a cell is determined primarily by the protein transformations originating in it (Nasonov and Aleksandrov, 1940; Aleksandrov, 1948). In connection with the specific peculiarities inherent to the given reaction, it is proposed to separate the conditions connected with it into a separate group under the common name of "cell stress."

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